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The Flavor and Fragrance High Production Volume Consortia

The Terpene Consortium

Test Plan for Estragole

Estragole **CAS No. 140-67-0**

FFHPVC Terpene Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

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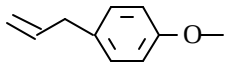
Test Plan for Estragole

1 IDENTITY OF SUBSTANCE



Estragole

CAS No. 140-67-0

Synonyms:

p-Allylanisole
Benzene, 1-methoxy-4-(2-propenyl)-
Chavicol methyl ether
Isoanethole
p-Methoxyallylbenzene
1-Methoxy-4-(2-propen-1-yl)benzene

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The terpene consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for terpene substances under the Chemical Right-to-Know Program. Twenty-one (21) companies are current members of the Terpene Consortium. The Terpene Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for estragole. Estragole is currently permitted by the U.S. Food and Drug Administration (FDA) for direct addition to food for human consumption as a flavoring substance and is considered by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel to be "generally recognized as safe" (GRAS) for its intended use as a flavoring substance [Hall and Oser, 1965]. Estragole occurs naturally in more than 39 foods [CIVO-TNO, 2000]. Exposure to estragole occurs principally though consumption of spices such as tarragon and essential oils derived from spices. Estragole is also added directly to food as a flavouring substance. Estragole modifies spice flavors and seasonings for condiments and meats. It is also used in heavy fruit, root beer, and anise-type

flavors. The estimated poundage of estragole added directly as a flavoring substance was reported to be approximately 500 kg [Lucas *et al.*, 1999].

Major sources of oral exposure occur *via* intake of basil, tarragon, anise, and bitter fennel. Greater than 90% of the mean daily *per capita* intake (1.0 micrograms/kg bw per day) of estragole is derived from consumption of tarragon, basil, fennel, anise and their essential oils. Based on the conservative assumption that only 10% of the U.S. population consumed foods containing estragole, the estimated daily *per capita* intake ("eaters only") of estragole from all sources is less than 10 micrograms/kg bw per day.

2.3 STRUCTURAL CLASSIFICATION

Estragole is 4-methoxyallylbenzene. Estragole is a C_{10} terpene that is recognized chemically as 4-methoxyallylbenzene. As a terpene derivative it is closely related in structure to other naturally occurring plant constituents containing a 4-alkoxyallylbenzene nucleus. Methyl eugenol (3,4-dimethoxyallylbenzene), elemicin (3,4,5-trimethoxyallylbenzene), myristicin (3-methoxy-4,5-methylenedioxyallylbenzene), and safrole (4,5-methylenedioxyallylbenzene) are all examples of *p*-alkoxyallylbenzene derivatives that can be found in spices such as nutmeg and basil. The only structural difference between estragole and these other alkoxyallylbenzene derivatives is the presence of additional ring alkoxy substituents (*i.e.*, methyl eugenol has a second ring methoxy group). *p*-Alkoxyallylbenzene derivatives participate in the same primary pathways of absorption, metabolism and excretion and exhibit the similar toxicologic endpoints (*i.e.* liver). Therefore, key data on *p*-alkoxyallylbenzene derivatives provide a more comprehensive chemical, biological and toxicological characterization of estragole.

Another structurally related substance is anethole. The structures of estragole and anethole (CAS No. 104-46-1) differ only in the position of the side-chain double bond. Estragole is 4-(2-propenyl)anisole while anethole is 4-(1-propenyl)anisole. Their similar physical properties reflect the small difference in chemical structure. The presence of an allyl side chain versus a 1-

propenyl side chain has an impact on the animal metabolism of each substance at high levels of exposure. Both substances are primarily detoxicated *via* *O*-demethylation at low levels of exposure (see below and the Test Plan for Anethole). At higher intake levels (greater than 50 to 100 mg/kg bw), estragole participates, to a significant extent, in a metabolic pathway (1'-hydrolylation) that, upon repeated daily exposure, is associated with hepatic toxicity. At these higher levels of intake, anethole mainly participates in a detoxication pathway (oxidative cleavage to yield a benzoic acid derivative) (see below). Therefore, human health toxicity data on anethole are considered relevant to estragole only in studies in which both substances participate in common pathways of metabolic detoxication (*e.g.*, *O*-demethylation) (see section 2.5 below).

2.4 INDUSTRIAL AND BIOGENIC PRODUCTION

The vast majority of estragole used as a flavoring agent in food is isolated from exotic (Reunion-type) basil that can contain as much as 90% estragole in the essential oil. Production of estragole from this source and other essential oils is approximately 10 metric tons annually [Bauer and Garbe, 1985]. However, the vast majority of estragole isolated from nature is as a component of crude sulfate turpentine (CST). Fractions containing estragole, anethole, and caryophyllene account for 1-2% of commonly distilled CST [Derefer and Traynor, 1992]. Although this represents only a small portion of CST, the sheer volume of production of CST on an annual basis provides the majority of estragole used for commercial purposes in food flavors, fragrances, cosmetics, and household products. Crude sulfate turpentine is fractionated into an anethole/caryophyllene mixture (0.5-1%) and an azetropic mixture of estragole and *alpha*-terpineol (1%). The majority of estragole present in this mixture is catalytically isomerized to anethole by the action of potassium hydroxide. The resulting mixture of anethole (mainly *trans*-anethole) and *alpha*-terpineol is further separated by fractional crystallization [Bauer and Garbe, 1985]. The majority of estragole isolated from CST, is converted to *trans*-anethole.

In 1977, it was reported that the annual production of CST in the United States was 92,750 tons (185,500,000 pounds). Based on the annual volume of production of CST and the

estragole content in CST (1%), it can be estimated that the potential amount of estragole isolated from CST is 1,855,000 pounds or 843,000 kg (843 metric tons).

Level III fugacity calculations indicate that, in the environment, estragole partitions mainly to the soil and water with less than 1% passing into the atmosphere. In the atmosphere, the relatively small amount of estragole rapidly reacts (half-life equals 3.9 hours) with hydroxyl radicals, ozone and nitrate radicals [Mackay, 1996a, 1996b]. Of more than 50 volatile organic compounds emitted by vegetation into the atmosphere, estragole was classified as exhibiting a relatively high rate of reactivity with hydroxyl radicals [Atkinson, 1990]. If it were conservatively assumed that 2% of industrially separated estragole is lost during industrial processing of CST, the vast amount (16.8 metric tons) would partition to the soil and water while the total annual estragole emitted into the atmospheric emission would be insignificant (0.17 metric tons). Compared to the amount (10 metric tons) consumed as a constituent of a traditional diet, exposure to industrial estragole loss to the atmosphere is insignificant. As a plant terpene, estragole is a normal component of the earth's atmosphere [Guenther *et al*., 1995]. However, in the absence of quantitative data on the emission rates of estragole from vegetation, it is not currently possible to estimate its annual rate biogenic production.

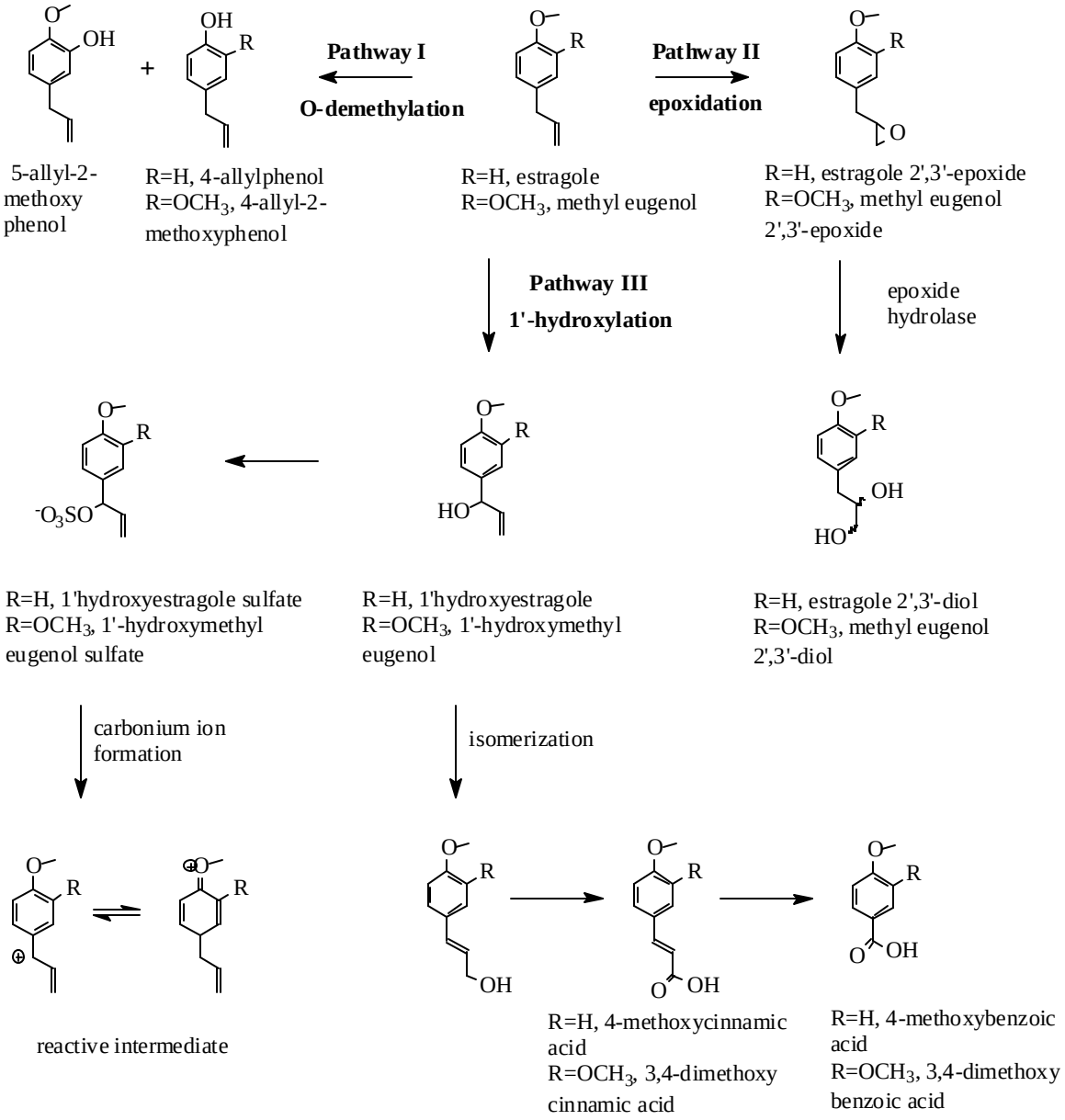
2.5 CHEMICAL REACTIVITY AND METABOLISM

The pharmacokinetic and metabolic pathways of estragole and methyl eugenol have been extensively reviewed in a recent publication (see Figure 1) [Smith *et al*., 2002]. Estragole undergoes rapid and essentially complete absorption *via* the oral route [Anthony *et al*., 1987; Sutton *et al*., 1985]. The metabolism of estragole and structurally related substances (*i.e*. methyl eugenol and *trans*-anethole) is clearly dose dependent. At low dose, (less than 10 mg/kg bw) estragole is primarily *O*-demethylated to yield the corresponding phenol derivative that is conjugated with glucuronic acid or sulfate and excreted mainly in the urine. Minor metabolic options at low levels of exposure include epoxidation of allyl double bond or 1'-hydroxylation at the benzylic position of the allyl side chain. As the dose is increased (0.05 to 1000 mg/kg bw) in

mice and rats, the *O*-demethylation pathway 1'-hydroxylation becomes saturated [Zangouras *et al*., 1981; Sangster *et al*., 1987]. The 1'-hydroxylation pathway has been shown to be a significant metabolic activation pathway leading to hepatotoxic effects in mice and rats [Miller *et al*., 1983; Phillips *et al*. 1981; Swanson *et al*., 1981; Wiseman *et al*., 1985]. Epoxidation of the allyl side chain yields a 2,3-epoxide that is detoxicated to the corresponding diol by epoxide hydrolase (EH) or to the corresponding mercapturic acid derivative by glutathione transferase (GST). Intoxication *via* the epoxidation of the allyl side chain is not as significant as activation *via* the 1'-hydroxylation pathway [Luo and Guenthner, 1995, 1996].

Intoxication *via* the 1'-hydroxylation pathway relies on formation of the labile sulfate conjugate (See Figure 1). The unstable sulfate ester hydrolyzes to form a reactive electrophilic intermediate (carbonium ion or quinonium cation) that binds hepatic proteins and hepatic DNA. The formation of protein and DNA adducts is dose-dependent [Drinkwater *et al.*, 1976; Miller *et al.*, 1982, 1983; Swanson *et al.*, 1981; Boberg *et al.*, 1983; Gardner *et al.*, 1995, 1996]. Studies on the inhibition of the sulfate conjugation pathway [Boberg *et al.*, 1983] and *in vivo-in vitro* unscheduled DNA synthesis (UDS) assays of estragole or methyl eugenol and their 1'-hydroxy metabolites [Chan and Caldwell, 1992; Caldwell *et al.*, 1992] provide substantial evidence that the sulfate ester of the 1'-hydroxy metabolite is the principal hepatotoxic metabolite in animals.

Figure 1
Metabolism of Estragole in Animals



*Excerpted from Smith *et al*., 2002

harmacokinetic Data

Approximately 70% of a 100 microgram dose of ^{14}C-methoxy-labeled estragole given by gelatin capsule to humans (2) was recovered within 48 hours, the majority of which was recovered in the urine (35% eliminated after 8 hours, 49.4% after 24 hours, and 61.2% after 48 hours), and remainder recovered in expired air (greater than 11% eliminated after 8 hours) [Sangster *et al.*, 1987].

In nine fasted human volunteers, ingestion of ginger snap cookies containing approximately 216 micrograms methyl eugenol (3.7 micrograms/kg bw) resulted in peak serum concentrations of 25-100 pg/g (approximately 0.000025-0.00010 micrograms/ml) with a mean of 16 pg/g [Masten, 2000]. A similar mean serum level (24 pg/g) was measured in 209 adults reported in an NHANES III U.S. survey. Over 98% of those surveyed containing detectable levels of methyl eugenol [Barr *et al*., 2000].

Serum levels in humans are 10,000 times less than those measured in mice and rats exposed to intoxicating levels of methyl eugenol [NTP, 2000; Graves and Runyon, 1995].

Greater than 95% of a single dose of 200 mg/kg bw of methyl eugenol or 56-66% of a dose of 100 mg/kg bw of estragole administered to male rats *via* gavage was excreted in the urine within 24 hours [Solheim and Scheline, 1973]. When the same dose levels were administered by intraperitoneal injection, greater than 85% of the methyl eugenol dose and 77-87% of the estragole dose were excreted in the urine after 24 hours [Solheim and Scheline, 1973].

In female rats, greater than 71% of a 50 mg/kg bw oral dose of ^{14}C-methoxy labeled estragole was eliminated in the first 24 hours with an additional 3.5% eliminated in the next 24 hours. Approximately 1% remained in the carcass at 48 hours. Approximately 38% was eliminated in the urine, 31% in expired air, and 1.3% in the feces [Zangouras, 1982]. In a dose-dependent

toxicokinetic study, female Wistar rats were given dose levels of 0.05 to 1000 mg/kg bw of ^{14}C-estragole. At the low doses (0.05 to 50 mg/kg bw), the majority (55% on day 1 and 2.7% on day 2) of the dose was eliminated as ^{14}C-labeled CO_2 in expired air. Urinary elimination accounted for a total 32.5% of the total radioactivity after 2 days. At higher dose levels (500 and 1000 mg/kg bw), elimination of radioactivity *via* expired air was less (29% on day 1 and 17% on day 2) and urinary elimination was greater (30% on day 1 and 29% on day 2) indicating a changeover in metabolism and elimination [Anthony *et al.*, 1987].

Rats administered either 37 or 150 mg/kg bw oral dose of methyl eugenol achieved peak plasma levels of 1.5 and 4 micrograms/ml. Plasma half-lives for methyl eugenol were 30 to 60 minutes and the area under the curve (AUC) was 97 and 225 micrograms/ml/minute at 37 and 150 mg/kg bw, respectively [Graves and Runyon, 1995].

F344/N rats (12/sex/group) were given 37 mg/kg bw by intravenous injection or 37, 75, or 150 mg/kg bw of methyl eugenol by oral intubation and blood was collected at time points up to 360 minutes [NTP, 2000]. Maximum plasma concentrations (C_{MAX}) of 0.656 to 3.84 micrograms/ml for males and 1.14 to 8.25 micrograms/ml for females were proportional to oral dose levels. Time to maximum plasma levels (T_{MAX}) was rapid (5 minutes) and independent of dose. The AUC increased linearly with dose for both males and females. The AUCs were in the range of 33.5 to 459 micrograms/ml/minute for males and 27 to 307 micrograms/ml/minute for females. Percent bioavailability also increased with dose. Bioavailability of methyl eugenol after a single oral dose was low (6% at 37 mg/kg bw and 19% at 150 and 300 mg/kg bw). Disappearance of half-lives were in the range from 60-115 minutes for both sexes. Seventy-two (72) hours after oral or intravenous administration of [^{14}C]-methyl eugenol to male rats, radioactivity was concentrated mainly in the liver (liver/blood ratio, 2-3) [NTP, 2000]. In mice given 25, 50, or 75 mg/kg bw, peak plasma levels were similar to those for rats (0.38 - 3.10 micrograms/ml for males and 0.12 - 4.4 micrograms/ml for females) and were reached in 5 minutes (T_{MAX}) in all groups except females in the 25 mg/kg bw groups which showed T_{MAX} of 15 minutes. Plasma half-lives were shorter (30 minutes) and the AUCs were significantly lower

than those recorded for rats (4.91-48.4 micrograms/ml/minute for males and 3.27-60.5 micrograms/ml/minute for females) indicating that methyl eugenol was eliminated more rapidly from the mouse.

In a second toxicokinetic study of longer duration [NTP, 2000], the pharmacokinetic profile was followed during repeated oral administration to rats and mice. Blood was taken from F344/N rats that had been treated with 37, 75, 150, or 300 mg/kg bw of methyl eugenol by gavage daily, 5 days per week for 6, 12, or 18 months. B6C3F1 mice treated at the same dose levels were monitored at 12 and 18 months. Absorption was extremely rapid in all dosed groups. Time to C_{MAX} was less than 5 minutes. Elimination from the blood was also rapid with elimination half-lives of 1-2 hours in both sexes. At 6 months, peak plasma levels (C_{MAX}) increased with increasing dose for most groups. Female concentrations (1.4-2.4 micrograms/ml) were higher than males (0.5-0.4 micrograms/ml) at the two lowest doses, but male concentrations (1.3-4.0 micrograms/ml) were higher than those (0.8-3.1 micrograms/ml) of females at the two highest doses. Generally, at the same dose levels, C_{MAX} was lower after 6 months of daily exposure than after single dose administration suggesting increased ability to metabolism methyl eugenol. Significant increases in both C_{MAX} and AUC between 6 and 12 months in the 150 and 300 mg/kg bw groups is evidence that metabolic saturation is achieved after prolonged exposure at higher dose levels. At all dose levels, females showed the AUC similar to naïve animals while males at 37, 75 and 150 mg/kg bw exhibited increased AUC suggesting enzymatic induction plays a more important role in males. An increase in the AUC with time suggests a decrease in the capacity to metabolize methyl eugenol with age [NTP, 2000].

For mice given 35, 75, or 150 mg/kg bw per day for 2 years, absorption was also rapid. C_{MAX} was reached after 5 minutes and increased with increasing dose for both male and females. Elimination half-lives increased with dose suggesting that the elimination was saturated for both sexes [NTP, 2000].

Male Fisher F344/N rats were given a single dose of 118 mg/kg bw [ring-^{14}C]-methyl eugenol and blood and urine were collected regularly and analyzed. Greater than 72% was eliminated in the urine, 13% in the feces, and less than 0.1% in expired air after 72 hours. Minute amounts (less than 0.4%) remained in the tissue at 72 hours with the majority being present in the liver. In female mice given the same dose, 85% was eliminated in the urine, 6% in the feces, less than 0.1% in the expired air, and less than 0.3% in the tissue. The largest amount was found in the fat, followed by the muscle and liver [Burkey *et al.*, 1999].

Based on the above data, it may be concluded that estragole and methyl eugenol are rapidly absorbed by the oral route and metabolized in the liver. Compared to female rats, male rats are more prone to experience metabolic saturation after prolonged (greater than 6 months), exposure to high dose levels of methyl eugenol. Male rats also experience metabolic induction at lower dose levels and earlier in exposure than do female rats.

In rodents and in humans, routes of elimination at low dose include loss of carbon dioxide *via* expired air (*i.e.*, arising from *O*-demethylation) and excretion of polar metabolites in the urine. At higher dose levels the fraction eliminated by expired air decreases while the fraction of non-volatile urinary metabolites increases.

2.5.1 Metabolism

Approximately 39% and 46% of a 100 mg/kg bw dose of estragole given to rats by the oral or intraperitoneal route, respectively, is present in the 48-hour pooled urine as the *O*-demethylation metabolite 4-allylphenol (See Figure 1). Other metabolites accounting for 17% of the oral dose or 31% of the intraperitoneal dose include the product of epoxidation, hydration and subsequent oxidation of the terminal alcohol (3-hydroxy-3-(4-methyoxyphenyl)propionic acid) of the allyl side-chain and the products of alkene isomerization, oxidation of the resulting C_3 position, and *beta*-oxidation yielding 4-methoxybenzoic acid and 4-methoxyhippuric acid. Approximately 5-

10% of the dose was excreted as the 1'-hydroxylation metabolite, 1'-hydroxyestragole [Solheim and Scheline, 1973].

A single intraperitoneal injection of 200 mg/kg bw of estragole, methyl eugenol, or safrole was given to male Wistar rats and urine was collected every 2 hours for 24 hours. Twenty-four (24) hours after treatment animals were terminated and the livers were removed. Urinary metabolites included the epoxide of the parent substance and the epoxide of the O-dealkylated metabolite (*i.e.*, *p*-allylcatechol epoxide from methyl eugenol and safrole and *p*-allylphenol epoxide from estragole). Liver homogenates showed the presence of safrole epoxide metabolites but not those of methyl eugenol or estragole. Liver microsomal preparations show the presence of the epoxide metabolite identified in the urine for all three substances [Delaforge *et al.*, 1980].

Twenty-one day old mice were given 185 micromoles/100 g bw of either estragole or safrole by intraperitoneal injection and the urine was analyzed for 1'-hydroxy metabolites 24 hours later. The dose level corresponds to 274 mg/kg bw of estragole and 300 mg/kg bw of safrole. Approximately 23% of estragole and 12% of the safrole was recovered from the 24-hour urine as the corresponding 1'-hydroxy metabolite, whereas, adult male mice (9-12 weeks) excreted up to 46% of the 300 mg/kg bw intraperitoneal dose of safrole as 1'-hydroxysafrole [Drinkwater *et al.*, 1976].

Formation of the 1'-hydroxy metabolite has been shown to be dose-dependent in both mice and rats [Zangouras *et al.*, 1981]. A dose-dependent increase in the urinary excretion of the glucuronic acid conjugate of 1'-hydroxyestragole occurs when dose levels of 0.05, 5, 500, 1,000 mg/kg bw of [^{14}C-methoxy]-estragole is administered orally to rats or by intraperitoneal injection to mice. Only 0.9% of the dose is excreted in the urine of rats given 0.05 mg/kg bw while 8.0% is found at 1,000 mg/kg bw. The total production and exposure to the 1'-hydroxy metabolite increased significantly (1,224 to 255,000 nmoles/kg per day) as the dose was increased from 5 to 500 mg/kg. Conversely, the same increase in dose resulted in a decrease in

O-demethylation from approximately 40% to 20% in both mice and rats. Thus, an increase in dose and a shift in metabolic pathways produce a marked increase in exposure to the 1'-hydroxy metabolite.

At low dose in humans, the 1'-hydroxylation pathway is of minor importance. Two male volunteers fed a gelatin capsule containing 100 micrograms [methoxy-^{14}C]-estragole (1.5 micrograms/kg bw) excrete the bulk (72% and 67%) of the radioactivity in the urine and as exhaled CO_2 within 48 hours. Principal metabolites included those derived from *O*-demethylation and oxidative degradation of the allyl side chain (i.e., 4-methyoxyhippuric acid, the glycine conjugate of 4-methoxycinnamic acid, and 4-methoxyphenyllactic acid). Urinary 1'-hydroxyestragole accounted for approximately 0.3% of the total dose [Sangster *et al.*, 1987]. The importance of *O*-demethylation pathway at low dose levels in human has also been observed for the double bond isomer, 4-propenylmethoxybenzene (anethole) [Sangster *et al.*, 1987; Caldwell and Sutton, 1988; Newberne *et al.,* 1999].

The 1'-hydroxylation pathway in rat and human liver microsomes indicate that the reaction is catalyzed predominantly by CYP2E1 and probably CYP2C6. The rate of 1'-hydroxylation of methyl eugenol varied widely in 13 human liver microsome samples (37 fold), but the highest activities in humans were similar to the activities in control rat liver microsomes [Gardner *et al.*, 1997]. Inducers of CYP-450 increased the number of methyl-eugenol-protein adducts. Auto-induction of the 1'-hydroxylation pathway was reported in hepatic microsomes of rats given 30-300 mg/kg bw per day oral doses of methyl eugenol for 5 days but not in rats given 10 mg/kg bw per day for 5 days [Gardner *et al.*, 1997].

In summary, *O*-demethylation is the principal detoxication pathway at low dose. At low dose levels, humans, mice, and rats show a similar tendency to metabolize alkoxyallylbenzene derivatives (*e.g.* estragole) by *O*-demethylation. At low dose significant amounts of estragole or methyl eugenol are *O*-demethylated, but as dose levels increase 1'-hydroxylation and

epoxidation of alkoxyallylbenzene derivatives (*e.g*. estragole) increase. Human production of 1'-hydroxy metabolite is expected to be very low levels of exposure (100 micrograms or 1.5 micrograms/kg bw) given that urinary excretion of the 1'-hydroxy metabolite accounts for less than 0.5% of urinary metabolites [Zangouras *et al*. 1981; Anthony *et al*., 1987].

2.6 SUMMARY FOR CATEGORY ANALYSIS

At low levels of exposure, estragole undergoes metabolic detoxication primarily *via* *O*-demethylation to yield the corresponding phenol derivative that is readily excreted as the glucuronic acid or sulfate conjugate in the urine. As dose levels increase, a switch in metabolism occurs in which an intoxication 1'-hydroxylation pathway competes favorably with the detoxication *O*-demethylation pathway. Under these high-dose conditions liver toxicity is normally observed in animal studies.

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

The calculated melting point for estragole has been reported to be -1.19 °C (adapted Stein and Brown method) [MPBPVP EPI Suite, 2000].

3.1.2 Boiling Point

The measured boiling point of estragole has been reported to be 216°C at 764 mm Hg [Merck Index, 1998] and 216°C at 760 mm Hg [Fragrance Materials Association]. The calculated boiling point according to the MPBPWIN program was 209.93°C at 760 mm Hg [MPBPVP EPI Suite, 2000]. Based on the consistency of these values, the boiling point of estragole is 216°C.

3.1.3 Vapor Pressure

Experimental value for vapor pressure was reported to be 1 mm Hg at 52.6°C [Stull, 1947]. The calculated vapor pressure of estragole has been reported to be 0.09 mm Hg (12 Pa) at 20°C [Fragrance Materials Association]. The vapor pressure of the isomer *trans*-anethole has been reported to be 0.05 mm Hg (6.67 Pa) at 20°C [FMA] and 0.041 (5.45 Pa) at 21°C for anethole, isomer unspecified [Daubert and Danner, 1989]. Given that the structure of estragole and anethole differ only in the position of a side-chain double bond, similar vapor pressures are expected at 20°C. Therefore, the vapor pressure of estragole is approximately 0.09 mm Hg (12 Pa) at 20°C.

3.1.4 n-Octanol/Water Partition Coefficients

The Log KOW was calculated resulting in a value of 3.47 [KOWWIN EPI Suite, 2000] for estragole, in good agreement with the log KOW of 3.39 [KOWWIN EPI Suite, 2000] and 3.11 [Interactive Analysis LogP and LogW Predictor] reported for the isomer anethole.

3.1.5 Water Solubility

The solubility of estragole in an experimental study was reported to be 178 mg/L at 25°C [WSKOWIN EPI Suite, 2000a (Yalkowski, S.H. and Dannenfelser, R.M., 1992)]. The calculated value based on the log KOW of 3.47 was reported to be 84.55 mg/L at 25°C [WSKOWIN EPI Suite, 2000b]. The water solubility of the double bond isomer anethole was reported to be 111 mg/L at 25°C that is in good agreement with the measured value for estragole [WSKOWIN EPI Suite, 2000a (Yalkowski and Dannenfelser, 1992)].

3.1.6 New Testing Required

None.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life value for estragole has been reported to be 2.36 hours [AOPWIN EPI Suite, 2000]. The short half-life of estragole is expected based on the fact that the 1'-position of the side chain is both a benzylic and an allylic position. This position is a site for rapid hydrogen abstraction by hydroxy radicals, peroxide radicals, and nitrogen dioxide radicals. Of more than 50 volatile organic compounds emitted by vegetation into the atmosphere, estragole was classified as exhibiting a relatively high rate of reactivity with hydroxyl radicals (no robust summary prepared) [Atkinson, 1990].

3.2.2 Stability In Water

No hydrolysis is possible for estragole. Estragole is expected to be stable in aqueous solution.

3.2.3 Biodegradation

The isomer of estragole, anethole, exhibited ready and ultimate biodegradability as measured by carbon dioxide production in an OECD 301B Guideline study. Anethole (mixed isomers) was 91% degraded within 28 days [Quest International Inc., 1994]. Based on model predictions [BIOWIN EPI Suite, 2000] estragole is anticipated to be ultimately biodegradable. Although model predictions and data available for the isomer, anethole, predict that estragole should be readily biodegradable, it is recommended that estragole be subjected to a biodegradability study according to a standard OECD Guideline protocol.

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model through the EPA EPI Suite 2000 program. The principal input parameters into the model are molecular weight (148.20), melting point (-1.19 °C), vapor pressure (0.09 mm Hg), water solubility (178 mg/L at 20 °C), and log Kow (3.47). The model predicts that estragole is distributed mainly to the soil (78.8%) and water (19.7%) with less than 1% passing into the atmosphere [Mackay, 1996a, 1996b].

The significance of these calculations must be evaluated in light of the fact that estragole is a product of plant biosynthesis. Therefore, the environment produces estragole. The model does not account for the influence of biogenic production on partitioning in the environment nor does it take into account any biodegradation.

3.2.5 New Testing Required

- Biodegradation study of estragole according to a standard OECD Guideline protocol.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

A measured LC50 is available for the *p*-alkoxyallyl derivative, methyl eugenol. In rainbow trout and bluegill sunfish, the 96-hour LC50 for methyl eugenol was determined to be 6 mg/L (95% C.I. 4.9-7.2 mg/L) and 8.1 mg/L (95% C.I. 7.4-9.0 mg/L), respectively [Beroza *et al*., 1975]. The acute 96-hour LC50 of anethole in fathead minnows using a continuous flow method was reported to be 7.69 mg/L [Broderius *et al.*, 1990]. Additionally, a calculated LC50 is available for estragole. The calculated 96-hour LC50 is 4.561 mg/L [ECOSAR EPI Suite, 2000].

Although the data for methyl eugenol, anethole and estragole consistently show an LC50 value of 5-10 mg/L, given the animal toxicity of estragole at high dose level, it is suggested that an LC50 be performed for estragole using a standard OECD Guideline 203 protocol.

3.3.2 Acute Toxicity to Aquatic Invertebrates

An OECD Guideline 202-I study is available for estragon oil (tarragon oil), the principal component of which is estragole (70-88%) (no robust summary for Lawrence, 1994). The 48-hour EC50 was 30.5 mg/L in *Daphnia magna* [Barth and Winkler, 2001]. The calculated 48-hour LC50 for estragole in *Daphnia magna* was reported to be 5.410 mg/L [ECOSAR EPI Suite, 2000]. This is in good agreement with an experimental 48-hour LC50 of 6.80 determined for *Daphnia magna* exposed to the 1-propenyl isomer, anethole [Broderius *et al.*, 1990].

3.3.3 Acute Toxicity to Aquatic Plants

The 96-hour IC50 experimental value for green algae for the structurally related substance *trans*-anethole was reported to be 9.571 mg/L [Broderius *et al*., 1990]. The calculated 96-hour EC50 for estragole in green algae was reported to be 3.681 mg/L [ECOSAR, EPI Suite,

2000]. Although the experimental IC50 value for the isomer is in good agreement with the calculated EC50 value for estragole, the calculated data should be further validated by comparison to an experimental EC50 determined for estragole. Therefore, an acute toxicity study is recommended using an OECD Guideline 202 protocol.

3.3.4 New Testing Required

Based on the current ecotoxicity database, the following studies are recommended:

- An acute toxicity study for fish using an OECD Guideline 203 protocol
- An acute toxicity study for algae using an OECD Guideline 201 protocol

3.4 HUMAN HEALTH TOXICITY

3.4.1 Acute Toxicity

In rats and mice, estragole showed low oral acute toxicity with oral LD50s of 1,230-1,820 mg/kg bw for rats and 1,250 mg/kg bw for mice. Low acute dermal toxicity is reflected in an LD50 value of greater than 5,000 mg/kg bw for rabbits [Moreno, 1972a, 1972b; Jenner *et al*., 1964].

Given the numerous studies available, additional acute toxicity tests in mammals are not recommended.

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

Extensive *in vitro* assays have been conducted on estragole and its metabolites. Estragole was negative in common strains of *Salmonella typhimurium* with and without metabolic activation [Zani *et al.*, 1991; Zeiger *et al.*, 1987; Sekizawa and Shibamoto, 1982; To *et al.*, 1982; Dorange *et al.*, 1977]. In one study [To *et al.*, 1982], a significant increase in the revertants per plate was reported for strain TA1538 in the presence of S-9 and 3'-phosphoadenosine 5'-phosphosulfate (PAPS) cofactor. The authors proposed that mutagenic response was related to the formation of the sulfate ester of an active metabolite. All other strains of *Salmonella typhimurium* were not mutagenic in assays using PAPS.

Other *in vitro* Ames assays with estragole and metabolites of estragole have produced equivocal results. Estragole was very weakly positive without metabolic activation in TA100 and positive in TA100 with activation. No effect was seen in TA98. The 2,3-epoxide of estragole and 1'-hydroxyestragole were positive in strains TA100 and TA1535, but negative in

TA98 with or without S-13 metabolic activation [Swanson *et al.,* 1979]. But in a different study no evidence of mutagenicity was reported when 1'-hydroxyestragole was incubated with strains TA98 and TA100 of *Salmonella typhimurium* with and without S-13 metabolic activation. Addition of PAPS as a cofactor did not induce an increase in revertants. 1'-Acetoxyestragole was mutagenic in strains TA98 and TA100 but not in a dose-dependent manner [Drinkwater *et al.,* 1976]. Overall, estragole and its 1'hydroxymetabolite do not appear to be mutagenic in *Salmonella typhimurium*.

Estragole concentrations of 0.001 to 0.00001 M did not induce the formation of chromosomal aberrations in V79 cells with and without metabolic activation or in primary rat hepatocytes [Muller *et al.,* 1994].

In an unscheduled DNA synthesis (UDS) study, a marked increase in UDS was reported when primary rat hepatocytes were incubated with estragole concentrations of 0.001 to 0.00001 M [Muller *et al.*, 1994]. When freshly prepared hepatocytes from Fisher F344 male rats were incubated with concentrations of estragole in the range from 0.000001 to 0.01 M, a significant increase in UDS, as much as 2.7 times control values, occurred at concentrations in the range from 0.0001 to 0.01 M [Chan and Caldwell, 1992]. Cytotoxicity was observed at concentrations in the range from 0.0001 to 0.01 M. Incubation of the 1'-hydroxyestragole showed increased UDS at concentrations greater than 0.00001 to 0.000001 M. Lactate dehydrogenase (LDH) leakage occurred at greater than 0.0001 to 0.00001 M for 1'-hydroxyestragole. The UDS activity and cytotoxicity of estragole occurred at concentrations approximately an order of magnitude greater than those for the 1'-hydroxy metabolites. Additionally, cytotoxicity was observed at slightly higher concentrations than those needed to induce UDS, although the differences were minimal. A clear non-linear relationship and threshold were established between dose for estragole or 1'hydroxyestragole and UDS activity. Similar results were obtained for estragole in an earlier study [Howes *et al.,* 1990].

3.4.2.2 In vivo

Several *in vivo* genotoxicity assays are available for estragole. In an *in vivo* UDS study, hepatocytes isolated 4 or 12 hours after rats received a 500, 1000, or 2,000 mg/kg bw dose of estragole were evaluated for unscheduled DNA synthesis. Very slight increases in net grain counts were reported at the 500 and 1,000 mg/kg bw dose, but only at 2,000 mg/kg bw dose were the net grain counts greater than 5, which was the criteria for a positive result [Muller *et al*., 1994].

In a study designed to detect DNA adduct formation of estragole and the 1'-hydroxyestragole metabolite, adult female CD-1 mice (mean weight 35 g) were given 12 micromoles/mouse (58 mg/kg) of [2',3'-^{3}H]-1'-hydroxyestragole by intraperitoneal injection in trioctanoin and DNA adduct formation monitored over 20 days post exposure. Similarly, 9-day old male or female B6C3F1 mice (mean weight, 6g) were given intraperitoneal injections of 0.5 micromoles (14 mg/kg) of labeled estragole and sacrificed after 23 hours. Three adducts were formed by the reaction of 1' or 3' positions (*cis* or *trans* isomers) of estragole with the exocyclic amino group (N^2) of deoxyguanosine. An additional adduct was formed by the reaction of the 3' position of estragole and the (N^6) position of deoxyadenosine. Unlike adducts of aromatic amines (*e.g.*, N-acetyl-2-aminofluorene) which persist at near maximum levels of binding for several weeks, the three adducts of estragole-deoxyribonucleoside were removed rapidly from mouse liver DNA. Timed measurement of DNA adducts indicated a biphasic loss indicated by a sharp decline in one of the two major 1'-hydroxyestragole adducts followed by relatively constant levels of liver DNA adducts from days 3 to 20, suggesting excision repair [Phillips *et al.*, 1981].

In ^{32}P-post-labelling experiments with adult female CD-1 mice (mean weight, 25 g) a 2 or 10 mg dose of estragole was given by intraperitoneal injection and liver DNA samples were collected 24 hours later. The dose levels in this study were equivalent to 100 or 500 mg/kg bw, respectively. Estragole show binding activities higher than allylbenzene, anethole, and other allyl substituted benzene derivatives. A rapid drop in total adduct formation occurred within 7 days

after dosing and was followed by a relatively constant level over the next 140 days, an effect also observed in the previous study. The authors noted that the significant decrease in DNA adduct levels was probably related to DNA repair processes [Randerath *et al.*, 1984].

In a related ^{32}P-post-labelling experiment [Phillips *et al.*, 1984], newborn male B6C3F1 mice were given 0.25, 0.5, 1.0, and 3.0 micromoles of alkoxybenzene derivatives (including estragole, methyl eugenol and safrole) by intraperitoneal injection on day 1, 8, 15, and 22, respectively, after birth. Dose levels on days 1 and 22 were estimated to be approximately 27 and 35 mg/kg bw, 1'-hydroxyestragole and 1'-hydroxysafrole, respectively. Mice were terminated on days 23, 29, and 43 and their liver DNA was isolated and analyzed. Highest DNA adduct levels were measured for methyl eugenol, estragole, and safrole compared to controls or other substances tested. A significant (p less than 0.05) amount of adduct was detected at 43 days. Based on the results of a study of carcinogenic activity of these substances in the same species and strain (see Miller *et al.*, 1983 in Repeat Dose Toxicity], the authors concluded that adduct levels of at least 15 pmoles/mg of DNA at 23 days were required for statistically significant tumor formation [Phillips *et al.*, 1984]. The authors also noted that, compared to adults, newborn mice showed greater sensitivity to alkenylbenzene carcinogenicity.

3.4.2.3 Conclusions

The genotoxicity database on estragole shows no mutagenic potential in the Ames assay. In cytogenetic assays, there is no evidence of a genotoxic potential *in vitro*. *In vitro* UDS studies showed positive responses when rat hepatocytes were incubated with estragole. In an *in vivo* study, UDS was seen at the 2,000 mg/kg bw dose and very weak responses were seen at the 500 and 1,000 mg/kg bw doses. As demonstrated by the studies on DNA adduct formation, estragole forms DNA adducts when laboratory rodents are exposed to high dose levels, so it is not surprising that both substances and their 1'-hydroxy metabolites induce unscheduled DNA synthesis. In these studies, concentrations at which UDS occurs coincide with hepatocellular cytotoxicity. Based on the available data, no additional genotoxicity tests are recommended.

3.4.3 Repeat Dose Toxicity

Groups of CD-1 female mice (mean weight 24 g) were maintained on a diet containing 2,300 or 4,600 ppm estragole or 2,500 ppm 1'-hydroxy estragole for 10 months. The authors estimated that the dietary levels corresponded to an average daily intake of 150-300 and 300-600 mg/kg bw for animals on the 2,300 ppm and 4,600 ppm estragole diet, respectively, and 180-360 mg/kg bw for animals on the 1'-hydroxyestragole diet. To avoid intolerance the dietary concentration was reduced by 75% for the first 10 days and 50% for the next 10 days. The target diet was then maintained for 12 months. Survival at 20 months was slightly lower (68-70%) for estragole fed animals compared to control animals (78%). The average life span of mice given 1'-hydroxyestagole was 13.6 months compared to 18 months in controls. Body weights measured at 1, 4, and 8 months were markedly reduced at 4 and 8 months compared to controls. At 10 months, the incidence of hepatomas was 58% for animals at 2,300 ppm estragole, 71% for animals at 4,600 ppm estragole and 56% for animals at 2,500 ppm of 1'-hydroxyestragole and 0 % in controls. Histopathological examinations revealed portal fibrosis, chronic inflammation and bile duct proliferation in addition to the tumors. Varied number of ceroid-laden histocytes and focal area of hyperplasia and megalocytosis were also reported. Four mice fed 4,600 ppm estragole had hepatic angiosarcomas [Miller *et al.*, 1983].

Additionally, CD-1 mice (male (55) and female (49)) were administered 370 mg/kg of estragole by gavage twice a week for ten doses beginning at 4 days of age. The mice were weaned at 35 days of age. Hepatomas were observed as early as 11 months. At 14 months, 73% of the males (3.5 hepatomas/mouse) and 24% of control males (0.6 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in females (9%, 0.1 hepatomas/mouse) was not statistically different from control females (2%, 0.02 hepatomas/mouse) [Miller *et al.*, 1983]. In another part of the study, male (50) and female (50) CD-1 mice were administered a total dose of 9.45 micromoles/mouse of estragole or estragole epoxide or 1.87 micromoles/mouse of 1'-hydroxyestragole by intraperitoneal injection distributed in a ratio of 1:2:4:8 on days 1, 8, 15, and 22, respectively, of life. These doses correspond to 0.63, 1.26, 2.52, and 5.04

micromoles/mouse, respectively. The mice were weaned at 22 days of age. At 12 months, 65% of the mice receiving estragole exhibited hepatomas (1.7 hepatomas/mouse) versus 26% of controls (0.5 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in mice given estragole epoxide (40%, 0.6 hepatomas/mouse) was not statistically different from control (26%, 0.5 hepatomas/mouse). For 1'-hydroxyestragole, 93% of the mice receiving the test substance (2.7 hepatomas/mouse) and 15% of control males (0.2 hepatomas/mouse) exhibited hepatomas [Miller *et al.*, 1983]

In a study using a hybrid strain of B6C3F1 mice, and the parent strain, C3H/He male and female mice and C57BL/6 male and female mice, the mice were given intraperitoneal injections of 1'-hydroxyestragole on days 1, 8, 15, and 22. Dose levels were 0.1 micromoles on day 1, 0.04 micromoles on days 8 and 15, and 0.08 micromoles on day 22 after birth. The levels are calculated to provide 11.7 on day 1, 18.8 on day 8, 9.3 on day 15 and 10.1 mg/kg bw on day 22, respectively. The experiment was terminated after 14 months. The first tumor-bearing mouse was observed at 10 months. At 12 months, 76% of the treated C3H/He male mice (3.0 hepatomas/mouse) and 26% of control mice (0.3 hepatomas/mouse) exhibited hepatomas. The incidence of hepatomas in C3H/He female mice, 6% (0.06 hepatomas/mouse), was not statistically different from those of control females. For C57BL/6 mice, the incidence of hepatomas in treated males was 14% (0.3 hepatomas/mouse) and was 5% (0.07 hepatomas/mouse) in control males. No hepatomas were observed in treated or control B57BL/6 female mice [Wiseman *et al.*, 1987].

In another part of the study, groups of male B6C3F1 mice were given single intraperitoneal injections of 0.10 micromoles/g (15 mg/kg) bw of 1'-hydroxyestragole or 1'-hydroxysafrole 12 days after birth. Animals were sacrificed after 12 months and incidence of hepatic tumors were measured. A second group of males was given a lower dose of 0.01 micromoles/g bw. A statistically significant increase in the incidence of hepatomas/mouse were observed for both substances at 0.1 micromoles/g bw, but no significant increase was observed at the low dose of 0.01 micromoles/g bw (1.5 mg/kg) [Wiseman *et al.*, 1987].

In a NTP carcinogenesis bioassay, male and female F344/N rats and male and female B6C3F1 mice were administered methyl eugenol in 0.5% methylcellulose by gavage daily at dose levels of 37, 75, or 150 mg/kg bw per day, five days per week for 2 years [NTP, 2000]. Stop-exposure groups of rats received 300 mg/kg doses for 53 weeks followed by the vehicle only (0.5% methylcellulose) for the duration of the study. All rats at the highest dose level (150 mg/kg bw) and the stop-exposure dose level (300 mg/kg bw) died before the end of the study. Mean body weights of all dosed groups were less than those of the vehicle controls throughout the study. The incidences of liver non-neoplastic lesions in dosed groups of male and female rats were increased at 6 months, 12 months, and 2 years. There were statistically significant increases in oval cell hyperplasia, hepatocyte hypertrophy, and eosinophilic foci, at all dose levels in male and female rats. At the three highest doses (75, 150, and 300 mg/kg bw per day) atypical focal bile duct hyperplasia, focal cystic degeneration, and mixed cell foci were observed, more in males than females. Many of the same non-neoplastic lesions of the liver were reported in the 300 mg/kg bw groups of male and female rats at both 6 and 12 months in the stop-exposure group. Non-neoplastic lesions of the glandular stomach included statistically significant increases in mucosal atrophy at all dose levels and neuroendocrine hyperplasia at the three highest dose levels in females and at all dose levels in males. There was a significant increase in the incidence of nephropathy in females at 300 mg/kg, and the incidence of renal tubule hyperplasia was greater in the greater than or equal to 75 mg/kg groups than in the vehicle control.

Liver neoplasms related to methyl eugenol exposure were reported in all dose groups and included hepatocellular adenomas and carcinomas, hepatocholangiomas, and In hepatocholangiocarcinomas. In all treated male and female rat groups, statistically significant increases (P equal to 0.049 in males and P equal to 0.017 in females at 37 mg/kg bw; P less than 0.001 for all other treated groups) in the incidence of hepatocellular adenomas and carcinomas were reported. Hepatocholangiomas and hepatocholangiocarcinomas were reported in the 150 mg/kg bw group of males (2/50, 4%) and females (3/49, 6%) and at higher incidence in the 300 mg/kg bw stop-exposure groups of males (13/50, 26%) and females

(17/50, 34%). Both benign (3/50, 6%) and malignant (4/50, 8%) neuroendocrine cell neoplasms of the glandular stomach were reported in males at 150 mg/kg bw and in the 300 mg/kg bw stop-exposure group (2/49, 4.1% benign and 2/49, 4.1% malignant). The incidence of these neoplasms was much higher in females at dose levels of 75 mg/kg bw (13/50, 26% benign and 12/50, 24% malignant) and greater. In male rats, there were significant increases in the incidence of: malignant mesothelioma at 150 mg/kg; mammary gland fibroadenoma at 75 and 150 mg/kg; and fibroma of the subcutaneous tissue at 37 and 75 mg/kg. These neoplasms were not found in female rats at any dose level.

For mice, survival of all male dosed groups was similar to that of the vehicle controls. The survival of treated female mice was significantly less than those reported for control animals. Mean body weights of dosed mice were reported to be "generally less than those of the vehicle controls throughout the studies". In female mice and, to a lesser extent, in male mice there was evidence of hepatotoxicity of methyl eugenol. Significant increases in oval cell hyperplasia, eosinophilic foci, hepatocyte hypertrophy and necrosis, haematopoietic cell proliferation, haemosiderin pigmentation, and bile duct cysts were observed at all dose levels in male and female mice. Non-neoplastic lesions of the glandular stomach included statistically significant increases in hyperplasia, ectasia, atrophy at all dose levels in both males and females and mineralization and necrosis in lower incidence also in both sexes incidences of chronic atrophic gastritis was high. Gastric tumors were found in two high dose males. The incidence of hepatocellular adenomas, hepatocellular carcinomas and hepatoblastomas was high in both treated and control male and female mice. While control males and females showed tumor rates of 63% (31/49) and 50% (25/50), respectively, and all treatment groups of males and females had tumor rates in excess of 92% with the exception of high dose male rates in which the tumor rate was 82% (41/50). Evidence of infection by *Heliobacter hepaticus* was found by PCR-RFLP, but associated hepatitis was not found.

An extensive interpretation [Smith *et al*., 2002] of the NTP study concludes that the study was compromised by a number of factors including malnutrition in both species, toxicity at all dose

levels, gastric damage affecting the absorption, distribution, and metabolism of methyl eugenol, and the presence of infection in both sexes of mice. Also, the authors conclude that the study cannot be recognized as conclusive for carcinogenicity at lower, non-toxic dose levels of methyl eugenol. According to the authors (robust summary not included):

"The methyl eugenol bioassay was compromised by inappropriately high dose levels, administered by gavage, that cause significant hepatotoxicity, gastric damage, and malnutrition in both mice and rats. The presence of Heliobacter hepaticus in the livers of mice was also thought to have confounded the interpretation of the findings. Hepatic tumors occurred in severely damaged livers while the neuroendocrine tumors were likely to have resulted from endocrine responses to chronic gastric damage. At dose levels of methyl eugenol at which hepatic tumors occurred in rats, non-neoplastic liver changes such as liver and hepatocyte enlargement, necrosis, chronic inflammation, periportal fibrosis and nodular or adenomatoid hyperplasia, were invariably present. Such recurrent liver damage, in particular chronic inflammation and hyperplasia undoubtedly altered methyl eugenol metabolism and may have strongly enhanced the likelihood of DNA damage, fixation of relevant DNA damage and progression of initiated/pre-neoplastic cells to cancer. Therefore, the hepatotoxicity induced by high dose levels of methyl eugenol most probably plays a very significant, if not an essential, role in the formation of hepatic tumors. If in humans, exposure to high levels of methyl eugenol were to be accompanied by recurrent liver tissue damage and hyperplasia, methyl eugenol might possibly induce liver cancer in humans. However, if dose levels of methyl eugenol in humans are less than those needed to induce hepatotoxicity (most probably somewhere in the range of 1 to 10 mg/kg bw/day), exposure of humans to such non-hepatotoxic levels can be assumed to be associated with a very low, probably zero, cancer risk."

3.4.4 Reproductive Toxicity

Studies are available for a mixture of *p*-allylalkoxybenzene derivatives in three different species at multiple dose levels [Morgareidge, 1973a, 1973b, 1973c] and for the isomer methyl eugenol [Le Bourhis, 1973].

In an FDA sponsored study [Morgareidge, 1973a, 1973b, 1973c] that evaluated both reproductive and developmental toxicity parameters, the essential oil of nutmeg containing a mixture of *p*-allylalkoxybenzene derivatives {myristicin, safrole, elemicin, and methyl eugenol

(10-20%)} and bicyclic terpene $C_{10}H_{16}$ hydrocarbons {*alpha*-pinene, *beta*-pinene, and sabinene (80-90%)} was given to pregnant CD-1 mice, Wistar rats, or golden hamsters.

In the mouse study, groups (20-21/group) of pregnant female CD-1 outbred mice were given 0, 6, 26, 120, or 560 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil on days 6 through day 15 of gestation. A positive control group received 150 mg/kg bw per day of aspirin. Maternal body weights were recorded on days 0, 6, 11, 15, and 17 of gestation. Females were observed daily for appearance and behavior. Food consumption and body weight were monitored to eliminate any abnormalities that may be associated with anorexia in pregnant females. On day 17 all dams were subjected to Caesarian section and the number of implantation sites, resorption sites, live fetuses, dead fetuses, and body weight of live pups were recorded. Gestation index, mortality, number of implantation sites, number of corpora lutea, litter size and weights, sex and sex ratio of pups, and gross abnormalities to pups were reported. The urogenital tract of each dam was examined for anatomical abnormalities. One-third of fetuses of each litter underwent detailed visceral examination at 10x magnification. The remaining two-thirds were stained with alizarin red S dye/KOH and examined for skeletal defects.

The administration of up to and including 560 mg/kg bw per day of test article FDA 71-28 to pregnant mice on days 6 through 15 of gestation had no effects on nidation, reproduction, maternal survival or any measured fetal parameter. The number and types of abnormalities seen in tissues of the dam or pups of the test groups did not differ for the number and type occurring spontaneously in the positive or negative controls.

The rat and hamster studies use the same study protocol as that used for the mouse study. Adult female Wistar or golden hamsters were individually housed in mess-bottom cages in a temperature- and humidity-controlled room. They were mated with untreated young adult males and observation of vaginal sperm plugs (rats) or appearance of motile sperm in vaginal smears

(hamsters) was considered day 0 of gestation. Groups (22-23/dose) of pregnant Wistar rats were then given 0, 3, 2, 56, or 260 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil daily on day 6 and through day 15 of gestation [Morgareidge, 1973c]. Groups (26-28/dose) of pregnant hamsters were given 0, 6, 28, 130, or 600 mg/kg bw of the test material (FDA 71-28) by gavage in corn oil daily on day 6 and through day 10 of gestation [Morgareidge, 1973b]. In the rats or hamster study, a positive control group received 250 mg/kg bw per day of aspirin.

The administration of up to and including 260 mg/kg bw per day of test article FDA 71-28 to pregnant rats on days 6 through 15 of gestation or administration of up to and including 600 mg/kg bw per day to pregnant golden hamsters on day 6 through day 10 of gestation had no effects on nidation, reproduction, maternal survival or any measured fetal parameter.

In the three-species study, no reproductive effects were observed when daily dose levels of up to 260 to 600 mg/kg bw of the essential oil predominantly composed of a combination of *p*-allylalkoxybenzene derivatives (10-20%) and bicyclic terpene hydrocarbons was administered daily to mice, rats, or hamsters during gestation. These dose levels correspond to dose levels of 26 to 120 mg/kg bw per day of *p*-allylalkoxybenzene derivatives. When this data is combined with the fact that no adverse effects were observed to the reproductive organs in 4-generation study with the double bond isomer anethole (see below), it is concluded that *p*-allylalkoxybenzene derivatives exhibit a low potential to produce reproductive toxicity.

In a comprehensive 4-generation study, groups of male and female rats (F_0) were fed 0 or 1% anethole in the diet (approximately 600-1,500 mg/kg bw per day) prior to mating, during the 15-day mating period, and during gestation and lactation. Offspring (F_1) were used for propagating the next generation and were raised on the same dietary treatment as their parents. A similar procedure was followed to obtain the 3rd and 4th generations (F_2 and F_3). The only notable effect was reduced body weight gain and body weights coinciding with reduced feed

intake in rats fed 1% anethole. There was no effect on reproductive performance over 4 generations. The reduced palatability of the diet was considered to be responsible for the lower body weight gain and body weights of the rats receiving anethole.

To ascertain the effect of palatability on the effects reported in the 4-generation study, a cross-fostering experiment was conducted using groups of control and treated F_1 females (from the 4-generation study and receiving 1% anethole in the diet) mated with control F_1 males (from the 4-generation study) [Le Bourhis, 1973]. Litters born from treated females were exchanged with litters from control females at birth and reared by the new dams. No significant difference in body weights of pups from those nursed by mothers of the same group, regardless from which group they were born, was reported and final body weights of pups born from treated dams but raised by control dams regained normal values by day 28. The results indicated that postnatal growth is not directly affected by anethole exposure, but is a result of the nutritional status of the dams [Le Bourhis, 1973].

Based on the results of reproductive toxicity on an essential oil containing a mixture of *p*-allylalkoxybenzene derivatives and an isomer anethole, no further testing on the possible reproductive toxicity of estragole is recommended.

3.4.5 Teratogenicity/Developmental Toxicity

A developmental study is available for the structurally related substance 4-methoxy-1-propenylbenzene (*trans*-anethole). In a developmental and reproductive screening test, groups of female rats were administered 0, 35, 175, or 350 mg anethole/kg bw per day *via* gavage in corn oil for 7 days prior to co-habitation with male rats until day 4 of lactation. The only notable effects were reduced mean body weights and decreased feed consumption in high-dose rats. These effects were seen to some extent in rats gavaged with anethole 175 mg/kg bw per day, but only reached statistical significance in the early part of the study. At the high dose (350 mg/kg bw per day), the number of liveborn pups was significantly decreased, the number of

stillborn pups was significantly increased, the number of pups dying on day 1 and days 2-4 was significantly increased, the viability index (number of live pups on postpartum day 4/number of liveborn pups on postpartum day 1) was significantly decreased, the number of surviving pups/litter on postpartum day 4 was significantly decreased, the live litter size on postpartum day 4 was significantly decreased, and pup weight/litter on postpartum day 1 was significantly decreased compared to controls. No anomalies and no other effects were reported. The authors determined the maternal and developmental no observable adverse effect level (NOAEL) to be 35 and 175 mg/kg bw per day, respectively, and the maternal and developmental lowest observable adverse effect level (LOAEL) to be 175 and 350 mg/kg bw per day, respectively. Anethole did not cause any effects on the rat fetus at doses below those causing maternal toxicity (reduced body weight and feed consumption).

In the FDA sponsored study discussed above [Morgareidge, 1973a, 1973b, 1973c], female pregnant CD-1 mice, Wistar rats, and golden hamsters were given dose levels of up 560, 260, and 600 mg/kg bw, respectively, of an essential oil containing 10-20% *p*-allylalkoxybenzene derivatives and 80-90% bicyclic terpene hydrocarbons daily by gavage during gestation. Based on clinical observations and measurement of body weight gain, mortality, and evaluation of the urogenital tract of pregnant females there were no signs of maternal toxicity at any dose level in any of the three species. Based on measurements of fetal survival, fetal body weight, visceral examination of pups, and a complete skeletal examination of pups at all dose levels, there was no evidence of developmental toxicity at any dose level in any of the three species.

Additionally, a developmental study is available for the related substance, safrole. No teratogenic effects were reported when safrole was administered intragastrically to female Swiss mice from days 6-14 of pregnancy [Moro *et al*., 1985].

Based on the lack of maternal and developmental toxicity in a four-generation study with the alkene isomer anethole, a developmental study with safrole, and a three-species study at

multiple dose levels of an essential oil containing a mixture containing *p*-allylalkoxybenzene derivatives [Morgareidge, 1973a, 1973b, 1973c], it is concluded that estragole is not a maternal or developmental toxicant.

No additional testing is recommended given the available data.

3.4.6 New Testing Required

None.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	**Boiling Point**	**Vapor Pressure**	**Partition Coefficient**	**Water Solubility**
Estragole CAS No. 140-67-0	Calc	A	A	Calc	A

Chemical	Environmental Fate and Pathways			
	Photodegradation	**Stability in Water**	**Biodegradation**	**Fugacity**
Estragole CAS No. 140-67-0	Calc	NA	R, Test	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
Estragole CAS No. 140-67-0	R, Test	R	R, Test

Chemical	Human Health Data					
	Acute Toxicity	**Genetic Toxicity *In Vitro***	**Genetic Toxicity *In Vivo***	**Repeat Dose Toxicity**	**Repro-ductive Toxicity**	**Develop-mental Toxicity**
Estragole CAS No. 140-67-0	A	A	A	A	R	R

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

4 REFERENCES FOR TEST PLAN AND ROBUST SUMMARIES

Anthony A., J.Caldwell, A.J.Hutt and R.L.Smith (1987) Metabolism of estragole in rat and mouse and influence of dose size on excretion of the proximate carcinogen 1'-hydroxyestragole. *Food and Chemical Toxicology*, 25(11), 799-806.

AOPWIN EPI Suite (2000) U.S. Environmental Protection Agency.

Atkinson R. (1990) Gas phase tropospheric chemistry of organic compounds: a review. *Atmospheric Environment*, 25A, 1063-1075.

Argus Research Laboratories, Inc. (ARL) (1992) Reproductive and developmental toxicity screening test of (anethole) administered orally *via* gavage to Crl:CDBR VAF/Plus female rats. Final Report.

American Society of Testing and Materials (ASTM) (1989) Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. E729. In: Vol. 11.04 of 1989 Annual Book of ASTM Standards. pp. 336-355.

Barr D.B., Barr J.R., Bailey S.L., Lapeza C.R., Jr., Beeson M.D., Caudill S.P., Maggio V.L., Schecter A., Masten S.A., Lucier G.W., Needham L.L. and Sampson E.J. (2000) Levels of methyleugenol in a subset of adults in the general U.S. population as determined by high resolution mass spectrometry. *Environmental Health Perspectives*, 108(4), 323-328.

Barth M. and Winkler, J (2001) Testing for acute toxicity of estragon oil (*Artemisia dracunculus L.*) in Daphne - *Daphnia magna*. Unpublished report.

Bauer K. and D. Garbe (1985) *Common Flavor and Fragrance Materials* Verlagsgesellschaft mbH, D-6940, Weinheim, Federal Republic of Germany.

Beroza M., Inscoe M., Schwartz P., Kepliknger M. and Mastri C. (1975) Toxicology and *Applied Pharmacology*, 31, 421-429.

BIOWIN EPI Suite (2000) U S Environmental Protection Agency.

Boberg E.W., E.C. Miller, J.A. Miller, A. Poland, and A. Liem. (1983) Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfooxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. *Cancer Research* 43(11), 5163-5173.

Broderius S., Hammermeister, D., Russom, C. (1990) Toxicity of eight terpenes to fathead minnows (*Pimephales promelas*), daphnids *(Daphnia magna*), and algae (*Selanastrum capriucornutum*). US EPA Environmental Research Laboratory/AScI Corporation. Unpublished.

Burkey J.L., Hoglen N.C., Kattnig M.J., Rice M.E. and Sipes I.G. (1999) The *in vivo* disposition and metabolism of methyleugenol in the Fischer 344 rat and the B6C3F1 mouse. *The Toxicologist*, 48(1-S), 224.

Caldwell J. and Sutton J.D. (1988) Influence of dose size on the disposition of trans-[methoxy-^{14}C]anethole in human volunteers. *Food and Chemical Toxicology* **26**, 87-91.

Caldwell J., V.S.W. Chan, A.D. Marshall, G. Hasheminejad, and S.V.J. Bounds. (1992) 1'-Hydroxylation is the only metabolic pathway of simple alkenylbenzenes involved in their genotoxicity. *The Toxicologist,* **12,** 56.

Chan V.S.W. and J. Caldwell. (1992) Comparative induction of unscheduled DNA synthesis in cultured rat hepatocytes by allylbenzenes and their 1'-hydroxy metabolites. *Food and Chemical Toxicology,* **30**, 831-836.

CIVO-TNO (2000) *Volatile Components in Food-Qualitative and Quantitative Data.* Supplement 5 to the 6th Edition. Edited by H. Maarse, C.A. Visscher, L.C. Willemsens, L.M. Nijssen, and M.H. Boelens. TNO Nutrition and Food Research. Zeist, The Netherlands.

Daubert T.E., and Danner, R.P. (1989) Physical and Thermodynamic Properties of Pure Chemicals: Data Compilation. Taylor and Francis, Washington, DC.

Delaforge M., P. Janiaud, P. Levi and J.P. Morizot (1980) Biotransformation of allylbenzene analogues in vivo and *in vitro* through the epoxide-diol pathway. *Xenobiotica* 10(10), 737-744.

Derfer and Traynor (1992) The Chemistry of Turpentine in "Naval Stores", Editors D. Zinkel and J. Russell.Pulp Chemicals Association. New York, New York.

Dorange J. L., Delaforge M. Janiaud P. and Padieu P. (1977) Mutagenicity of the metabolites of the epoxide diol pathway of safrole and analogs. Study on Salmonella typhimurium. Societe de Biologie de Dijon, 171(5), 1041-1048.

Drinkwater N.R., E.C. Miller, J.A. Miller, and H.C. Pitot. (1976) Hepatocarcinogenicity of estragole (1-allyl-4-methoxybenzene) and 1'-hydroxyestragole in the mouse and mutagenicity of 1'-acetoestragole in bacteria. *Journal of the National Cancer Institute,* 57, 1323-1331.

ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Fragrance Materials Association (FMA). Reported values for boiling point for estragole. Unpublished report.

Fragrance Materials Association (FMA). Reported values for vapor pressure for estragole. Unpublished report.

Fragrance Materials Association (FMA). Reported values for vapor pressure for *trans*-anethole. Unpublished report.

Gardner I., P. Bergin, P. Stening, J.G. Kenna, and J. Caldwell (1995) Protein adducts derived from methyleugenol. ISSX International Meeting, 4th, Vol. 8: 208.

Gardner I., P. Bergin, P. Stening, J.G. Kenna, and J. Caldwell (1996) Immunochemical detection of covalently modified protein adducts in livers of rats treated with methyleugenol. *Critical Reviews in Toxicology*, 9(4), 713-721.

Gardner, I. B., I. Blench, H.R. Morris, J. Caldwell, and J.G. Kenna. (1997) Covalent modification of the laminin receptor precursor protein by reactive metabolites of methyleugenol. ISSX International Meeting, 6th, Vol. 11, 244.

Graves S W. and Runyon S. (1995) Determination of methyleugenol in rodent plasma by high-performance liquid chromatography. *Journal of Chromatography B: Biomedical Applications*, 663(2), 255-262.

Guenther A., Hewitt, C., Erickson, D., Fall, R., Geron, C., Graedel, T., Harley, P., Klinger, L., and M. Lerdau (1995) A global model of natural volatile organic compound emissions. *J. Geophys. Res, Atmos.*, 100, D5: 8873-8892.

Hall R.L. and Oser, B.L. (1965) Recent progress in the consideration of flavoring ingredients under the food additives amendment. III. GRAS substances. *Food Technology* 19:151-197.

Howes A.J., Chan V.S.W. and Caldwell J. (1990) Structure-specificity of the genotoxicity of some naturally occurring alkenylbenzenes determined by the unscheduled DNA synthesis assay in rat hepatocytes. *Food and Chemical Toxicology*, **28(8),** 537-542.

Interactive Analysis LogP and LogW Predictor: Database contributed by Syracuse Research Corporation, SciVision, Albany Molecular Research, Inc., eduSoft LC, Cambridge Soft. www.logp.com.

Jenner P.M., Hagan E.C., Taylor J.M., Cook E.L. and Fitzhugh O.G. (1964) Food flavorings and compounds of related structure I. Acute oral toxicity. *Food and Cosmetics Toxicology*, 2(3), 327-343.

Klimisch H. J., Andreae, M., and U. Tillman (1997) A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Journal of Regulatory Toxicology and Pharmacology*, 25, 1-5.

KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency.

Lawrence B.M., R.J. Reynolds Tobacco Co., Winston-Salem, NC (1994) Is the development of an essential oil industry in Malaysia a viable commercial opportunity? Edit. by Lawence B. M. pp. 187-204, Allured Publishing Corporation Carol Stream, Illinois.

Le Bourhis B. (1973) 4-Generation reproduction study in rats given trans-anethole in the diet. Unpublished report by Sophie Holm. Laboratoire de Physiologie, Institut de Recherches appliquees aux Boissons, Montreuil, 93, France.

Luo G. and Guenthner, T.M. (1995) Metabolism of allylbenzene 2',3'-oxide and estragole 2',3'-oxide in the isolated perfused rat liver. *The Journal of Pharmacology and Experimental Therapeutics*, **272(2),** 588-596.

Luo G. and Guenthner, T.M. (1996) Covalent binding to DNA in vitro of 2',3'-oxides derived from allylbenzene analogs. *Drug Metabolism and Disposition*, 24(9), 1020-1027.

Lucas C.D., Putman J.M., and Hallagan J.B. (1999) Flavor and Extract Manufacturers Association (FEMA) of the United States 1995 poundage and technical effects update survey. Washington, DC. Unpublished report.

Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, 15(9), 1618-1626.

Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, 15(9), 1627-1637.

Margolin B.H., Kaplan N., Zeiger E. (1981) Statistical analysis of the AMES Salmonella/microsome test. Proc National Acad Sci USA 78, 3779-3783.

Margolin B.H, Resnick M.A., Rimpo J.Y., Archer P., Galloway S.M., Bloom A.D., Zeiger A.D. (1986) Statistical analysisfor in vitro cytogenetic assays using Chinese hamster ovary cells. Environ Mutagen 8, 183-204.

Masten S. (2000) Private communications to FEMA. Unpublished report.

Merck Index (1998) The Merck Index. Merck & Co., Inc. Whitehouse Station, NJ.

Meyer B.N., Ferrigni N.R., Putnam J.E., Jacobsen L.B., Nichols D.E., Moore D.S., McLaughlin J.L. (1982) Planta Med 45: 31-34.

Miller J.A., E.C. Miller and D.H. Phillips. (1982) The metabolic activation and carcinogenicity of alkenylbenzenes that occur naturally in many spices. *Carcinogens and mutagens in the Environment*, 1, 83-96.

Miller E.C., A.B. Swanson, D.H. Phillips, T.L. Fletcher, A. Liem, and J.A. Miller. (1983) Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer Research*, **43,** 1124-1134.

Moreno O. (1972a) Acute dermal toxicity of estragole in rabbits. Unpublished report to RIFM.

Moreno O. (1972b) Acute oral toxicity of estragole in rats. Unpublished report to RIFM.

Morgareidge K. (1973a) Teratologic evaluation of FDA 71-28 in mice. Contract No. FDA 71-260. Unpublished report.

Morgareidge K. (1973b) Teratologic evaluation of FDA 71-28 in hamsters. Contract No. FDA 71-260. Unpublished report.

Morgareidge K. (1973c) Teratologic evaluation of FDA 71-28 in rats. Contract No. FDA 71-260. Unpublished report.

Moro M.G., Ognio E., Rossi L. et al. (1985) Prenatal toxicity of safrole in laboratory animals. *Riv. Tossicol. Sper. Clin*. (Italy), **15/1-2**, 91-97.

MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

Muller L. Kasper P., Muller-Tegethoff K. and Petr T. (1994) The genotoxic potential *in vitro* and *in vivo* of the allyl benzene etheric oils estragole, basil oil and *trans*-anethole. *Mutation Research*, **325(4),** 129-136.

Nabholz V. and G. Cash (1998) ECOSAR, U.S. Environmental Protection Agency, OPPT Risk Assessment Division.

National Toxicology Program (NTP). (2000) Toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-12) in F344/n rats and B6C3F1 mice (gavage studies). DRAFT NTP-TR-491; NIH Publication No. 98-3950.

Newberne P., Smith R.L., Doull J. Goodman J.I., Munro I.C. Portoghese P.S. Wagner B.M., Weil C.S., Woods L.A., Adams T.B., Lucas C.D. and Ford R.A. (1999) The FEMA GRAS assessment of trans-Anethole Used as a Flavouring Substance. *Food and Chemical Toxicology*, 37, 789-811.

Phillips, D.H., J.A. Miller, E.C. Miller, and B. Adams. (1981) Structures of the DNA adducts formed in mouse liver after administration of the proximate hepatocarcinogen 1'-hydroxyestragole. *Cancer Research*, **41,** 176-186.

Phillips D.H., Reddy M.V. and Randerath K. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally occurring alkenylbenzenes. II. Newborn male B6C3F1 mice. *Carcinogenesis*, **5(12),**

1623-1628.

Quest International, Inc. (1994) The ultimate and readily biodegradation of anethole. Unpublished report.

Randerath K., Haglund R.E., Phillips D.H. and Reddy M.V. (1984) 32P-Post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occuring alkenylbenzenes. I. Adult female CD-1 mice. *Carcinogenesis,* **5(12),** 1613-1622.

Sangster S.A., J. Caldwell, A.J. Hutt, A. Anthony, and R.L. Smith. (1987) The metabolic disposition of [methoxy-14C]-labeled trans-anethole, estragole and p-propylanisole in human volunteers. *Xenobiotica,* **17**, 1223-1232.

Sekizawa J. and Shibamoto T. (1982) Genotoxicity of safrole-related chemicals in microbial test systems. *Mutation Research,* **101(1),** 127-140.

Smith R. L., T. B. Adams, J. Doull, V.J. Feron, J.I. Goodman, L.J. Marnett, P. S. Portoghese, W. J. Waddell, B. M. Wagner, A. E. Rogers, J. Caldwell, and I. G. Sipes (2002) Safety Assessment of Allylalkoxybenzene Derivatives Used as Flavouring Substances- Methyl eugenol and Estragole. Flavor and Extract Manufacturers' Association, Washington, D.C. Unpublished report.

Solheim E. and Scheline R.R. (1973) Metabolism of alkenebenzene derivatives in the rat. II. Eugenol and isoeugenol methyl ethers. *Xenobiotica,* 6(3), 137-150.

Stull D.R. (1947) Vapor pressure of pure substances. Organic Compounds. *Ind Eng Chem*, 39, 517-540.

Sutton J.D., S.A. Sangster, and J. Caldwell. (1985) Dose-dependent variation in the disposition of eugenol in rat. *Biochemical Pharmacology,* 34, 465-466.

Swanson A.B., Chambliss D.D., Blomquist J.C., Miller E.C. and Miller J.A. (1979) The mutagenicities of safrole, estrogole, eugenol, trans-anethole, and some of their known or possible metabolites for Salmonella typhimurium mutants. *Mutation Research,* **60(2),** 142-153.

Swanson, A.B., E.C. Miller, and J.A. Miller. (1981) The side-chain epoxidation and hydroxylation of the hepatocarcinogens safrole and estragole and some related compounds by rat and mouse liver microsomes. *Biochimica et Biophysica Acta,* 673, 504-516.

Syracuse Research Corporation (SRC). Private communication to FMA. Unpublished report.

To L.P., Hunt T.P. and Andersen M.E. (1982) Mutagenicity of trans-anethole, estragole, eugenol and safrole in the Ames Salmonella typhimurium assay. Bulletin of Environmental Contamination and Toxicology, 28(6), 647-654.

Wiseman R.W., Fennell T.R., Miller J.A. and Miller E.C. (1985) Further characterization of the DNA adducts formed by electrophilic esters of the hepatocarcinogens 1'-hydroxysafrole and 1'-hydroxyestragole in vitro and in mouse liver in vivo, including new adducts at C-8 and N-7 of guanine residues. *Cancer Research*, 45, 3096-3105.

Wiseman R.W., Miller E.C., Miller J.A. and Liem A. (1987) Structure-activity studies of the hepatocarcinogenicities of alkenylbenzene derivatives related to estragole and safrole on administration to preweanling male C57BL/6J x C3H/HeJ F1 mice. *Cancer Research*, 47(9), 2275-2283.

WSKOWIN EPI Suite (2000a) U S Environmental Protection Agency (Yalkowski S.H., and Dannenfelser, R.M., 1992)

WSKOWIN EPI Suite (2000b) U S Environmental Protection Agency.

Zangouras, A., J. Caldwell, A.J. Hutt, and R.L. Smith. (1981) Dose dependent conversion of estragole in the rat and mouse to the carcinogenic metabolite, 1'-hydroxyestragole. *Biochemical Pharmacology,* 30, 1383-1386.

Zangouras A. (1982) Doctorial dissertation: Metabolic studies in the assessment of the human carcinogenic hazard of estragole. Unpublished.

Zani F., Massimo G., Benvenuti S., Bianchi A., Albasini A., Melegari M., Vampa G., Bellotti A., Mazza P. (1991) Studies on the genotoxic properties of essential oils with *Bacillus subtilis* rec-assay and Salmonella microsome reversion assay. *Planta Medica* **57**(3), 237-241.

Zeiger E, Anderson B., Haworth S. Lawlor T., Mortelmans K. and Speck W. (1987) Salmonella mutagenicity tests: III. Results from testing 255 chemicals. *Environmental Mutagenesis* **9(9**), 1-109.